

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

These amendments introduce no new matter and support for the amendment is replete throughout the specification and claims as originally filed. These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter, or agreement with any objection or rejection of record.

Listing of Claims:

Claims 1 to 33 (Cancelled).

34. (Currently amended) A method of incorporating a 5-substituted tryptophan unnatural amino acid into a peptide, the method comprising:

preparing a construct comprising a nucleic acid sequence encoding an orthogonal mutant tryptophanyl-tRNA synthetase (O-muTrpRS) and comprising at least 90% identity to the sequence of SEQ ID NO: 2 ~~or a conservative variant thereof~~, the O-muTrpRS comprising a proline residue at a position corresponding to position 144 of SEQ ID NO: 2;

preparing a construct comprising a nucleic acid sequence encoding an orthogonal tRNA (O-tRNA) comprising: at least 90% identity to SEQ ID NO: 3, a suppressor anticodon and a eukaryotic A box transcriptional control element;

introducing the O-muTrpRS construct and the O-tRNA construct into a eukaryotic cell; and,

preferentially aminoacylating an expressed O-tRNA with the unnatural amino acid, wherein said aminoacylation is catalyzed by an expressed O-muTrpRS;

whereby the 5-substituted tryptophan unnatural amino acid is incorporated into the peptide in the cell.

35. (Currently amended) The method of claim 34, wherein the unnatural amino acid comprises a ~~tryptophan analog or~~ 5-hydroxy-L-tryptophan (5-HTPP).

36. (Original) The method of claim 35, further comprising applying a voltage to the peptide, thereby reacting the 5-HTPP with a reactive molecule.

37. (Original) The method of claim 36, wherein reacting comprises cross-linking.

38. (Original) The method of claim 36, wherein the reactive molecule comprises an unnatural amino acid in another peptide.

39. (Original) The method of claim 34, further comprising detecting an interaction between the peptide and another peptide.

40. (Original) The method of claim 39, wherein said detecting comprises fluoroscopy.

41. (Previously presented) The method of claim 34, wherein the O-muTrpRS construct comprises a nucleic acid comprising a polynucleotide sequence selected from the group consisting of:

- a) a coding polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, and a conservative variation thereof;
- b) a coding polynucleotide sequence that encodes a polypeptide selected from the group consisting of SEQ ID NO: 2, and a conservative substitution thereof;
- c) a polynucleotide sequence which hybridizes under highly stringent conditions over an entire length of a polynucleotide sequence of (a) or (b);
- d) a complementary sequence of (a), (b), or (c); and,
- e) a Val144Pro mutant of plasmid pEF6-TrpRS (pVal144ProBsTrpRS).

42. (Original) The method of claim 34, wherein the O-muTrpRS construct comprises a mutated tryptophanyl-tRNA synthetase peptide sequence mutated at one or more amino acid residues based on structure data of the tryptophanyl-tRNA synthetase or an analogous aminoacyl-tRNA synthetase.

43. (Original) The method of claim 42, wherein the mutated tryptophanyl-tRNA synthetase comprises a *Bacillus* tryptophanyl-tRNA synthetase mutated at Val144.

44. (Currently amended) The method of claim 34, wherein the O-tRNA construct comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, a ~~conservative variation thereof~~, and a complementary polynucleotide sequence thereof.

45. (Original) The method of claim 34, wherein said preparing the O-tRNA construct comprises inclusion of one or more tRNA flanking sequences that functionally interact with an RNA polymerase of the cell.

46. (Cancelled)

47. (Currently amended) The method of claim 34, further comprising mutating a prokaryotic tRNA sequence to include ~~at~~the functional A box eukaryotic transcriptional control element.

48. (Original) The method of claim 47, wherein said mutating comprises site directed mutagenesis.

49. (Original) The method of claim 34, wherein the O-tRNA construct or O-muTrpRS construct comprises: a reporter tag or a purification tag.

50. (Original) The method of claim 34, wherein the O-muTrpRS construct and the O-tRNA construct comprise the same construct.

51. (Original) The method of claim 34, wherein the O-tRNA recognizes a selector codon in a nucleic acid sequence encoding the peptide, thereby incorporating the unnatural amino acid into the peptide.

52. (Original) The method of claim 34, further comprising transfected a nucleic acid encoding the peptide into the cell.

53. (Original) The method of claim 52, wherein the cell comprises a eukaryotic cell or mammalian cell.

54. (Original) The method of claim 34, further comprising expressing the O-muTrpRS construct or the O-tRNA construct.

55. (Original) The method of claim 54, further comprising purifying expressed O-muTrpRS or expressed O-tRNA.

Claims 56 to 62 (Cancelled).

63. (Previously presented) The method of claim 34, wherein the O-muTrpRS comprises at least 95% identity to SEQ ID NO: 2.

64. (Previously presented) The method of claim 34, wherein the O-muTrpRS comprises at least 98% identity to SEQ ID NO: 2.

65. (Previously presented) The method of claim 34, further comprising mutating and screening a nucleic acid encoding the amino acid sequence of SEQ ID NO: 2 to obtain the O-muTrpRS.

66. (Currently amended) The method of claim 34, wherein the O-muTrpRS comprises with two adjacent binding pockets separated by an a-helix peptide consisting of Asp at a position corresponding to position 140, Ile at a position corresponding to position 141, Val at a position corresponding to position 142, Pro at a position corresponding to position 143, ~~an amino acid other than Val at a position corresponding to position 144~~, and Gly at a position corresponding to position 145.

67. (Previously presented) The method of claim 34, wherein the O-muTrpRS comprises Ser at a position corresponding to position 7, His at a position corresponding to position 44, and Asp at a position corresponding to position 133.

68. (New) The method of claim 34, wherein the A box eukaryotic transcriptional control element comprises: G at a position corresponding to position 7, G at a position corresponding to position 9, or U at a position corresponding to position 11.

69. (New) The method of claim 34, wherein the O-tRNA comprises at least 95% identity to the sequence of SEQ ID NO: 3.